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PENETRATION OF 5-FLUOROURACIL IN EXCISED SKIN*

JORDAN L. COHEN, PH.D.†, AND RICHARD B. STOUGHTON, M.D.‡

ABSTRACT

Total penetration of 5-fluorouracil (FU) through human and hairless mouse skin was measured in vitro and the results were compared using radioactively labelled drug and a gas chromatographic method specific for the free FU molecule. Both methods were used to determine whether metabolism, either in the skin during penetration or in the penetration wells, could have affected the percent penetration as calculated by the radioactivity method. The results indicate significantly greater drug penetration in the mouse samples using both methods of detection, with somewhat greater variations between identical samples occurring with the chromatographic method. The percent penetrating the human samples, approximately 12 percent after 20 hr, agreed with the previously reported in vivo data. These studies indicate that further in vitro studies may be useful to study the factors affecting the penetration of FU when applied topically in a variety of skin diseases.

5-fluorouracil has been demonstrated to have significant biologic activity when applied topically in a wide variety of skin diseases. Nurse [1] was the first to report evidence of activity of several antimetabolites, including FU, on epidermal structures. Soon thereafter topical application of FU was shown to be effective in actinic keratoses [2] and epithelial neoplasms [3]. A relatively recent report [4] indicates satisfactory control of psoriatic plaques following topical FU therapy.

To date, only a single study by Dillaha et al [5] has been directed towards the percutaneous absorption of FU. This study utilized radiolabelled drug and measured excretion of radioactivity in the urine following topical application. Since FU is known to be converted in vivo to the nucleoside and nucleotide, which still retain the ^{14}C label [6, 7], this is a relatively nonspecific determination which could not account for metabolic alteration of the drug at the site of absorption.

This report describes the comparison of penetration of FU through human and hairless mouse skin in vitro using radiolabelled drug and subsequent liquid scintillation counting, and a gas–liquid chromatographic method of analysis specific for the free FU molecule. Differences between the two methods were not found to be statistically significant and the percent penetration of FU in the human skin samples agreed with the results by Dillaha [5].

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* From the School of Pharmacy, University of Southern California, Los Angeles, and ‡the Division of Dermatology, Scripps Clinic and Research Foundation, La Jolla, California. † Present address: Virginia Commonwealth University, School of Pharmacy, MCV Station, Richmond, Virginia 23298.

MATERIALS AND METHODS

Reagents. All chemicals and solvents used were of analytical reagent-grade and water was glass-double-distilled.

Preparation of excised skin samples. Undamaged human skin was obtained from the upper areas of surgically amputated legs, while hairless mouse skin was obtained from the backs of 2- to 4-month-old animals which had been sacrificed by ether inhalation. The tissues were cut into portions and stored at $-17°$ to $-22°$ C in airtight containers. They were thawed at room temperature 1 h prior to use. The fat was carefully dissected from the bottom of the dermis of the human skin samples which were then applied dermis side down to a glass penetration well as described in a previous paper [8]. Hairless mouse skin was used without dissection since it easily separated from the underlying muscle and there was little subcutaneous fat. This was applied dermis side down to the glass wells in the same manner as the human skin samples.

All specimens were placed in a chamber controlled at 33° C dry bulb and 30° C wet bulb. The wells were filled with normal saline which bathed the dermal side of the skin samples, and open plastic containers (cylinders), 1.5 cm in diameter, were attached to the epidermis by Duco® cement. 0.01–0.02 ml of an appropriate FU solution in propylene glycol was added to the skin in these plastic containers.

Aliquots of the solution in the wells bathing the dermis were taken at regular intervals over a 24-hr period and analyzed for FU using one of the methods described below. After each withdrawal, an equal volume of fresh saline was replaced in the wells and the FU penetration was calculated over the time interval.

Radioactivity studies. 5-fluorouracil-2^{14}C (International Chemical and Nuclear, Irvine, California; spec. act. 25 mc/mM) was added to propylene glycol along with unlabelled FU to make a 4% solution with approximately 10×10^6 disintegrations/min/.01 ml of solution. 0.01–0.02 ml of this solution was applied to the skin samples. 1.0-ml aliquots were taken from the penetration wells at specific intervals and diluted with Bray's solution for subsequent liquid scintillation counting. Standard procedures were used to determine the quenching of the samples.

Gas chromatographic studies. 0.01–0.02 ml of a 4% solution of FU in propylene glycol was applied to the skin

samples as described earlier. Aliquots were removed at the appropriate intervals and subjected to gas chromatographic analysis. This involves the extraction of the free FU from the aqueous samples into a propanol-ether mixture, reaction with a derivitizing reagent§ to form the di-trimethylsily derivative, followed by quantitation using a flame ionization detector and an internal standard. Samples may be collected and frozen until ready for analysis. The details of the procedure appear elsewhere [9].

RESULTS

Table I indicates the results from penetration studies applying 0.02 ml of a 4% solution of FU to 4 human skin and 4 hairless mouse samples. Aliquots were removed from the penetration wells between 30 min and 24 hr and were analyzed by either the liquid scintillation method (LSC) or the gas chromatographic method (GLC). The cumulative percent penetrated at each time represents the mean of the four samples. Table II compares the penetration of 0.01 ml of a 4% solution applied to 8 human skin and 5 mouse samples after 20 hr.

For comparative purposes with other molecules, Table III expresses the mean 20-hr data as molar transepidermal fluxes of FU per unit time.

DISCUSSION

The results indicate fairly high penetration of FU through excised human and hairless mouse skin as evidenced by both methods of detection. The rate of penetration of FU was of the same order of magnitude in both species, but the total penetration was significantly higher in the mouse skin samples. The percent penetration is of the same order of magnitude as that calculated by Dillaha [5] from urinary excretion data following topical application of FU-2-^{14}C on five patients. He had calculated a 10–15 percent penetration which agrees well with our values of 13 percent and 11 percent calculated by the two different analytical methods. The in vivo penetration must be viewed cautiously, however, because of the large reported differences in the urinary excretion of total radioactivity [10] following FU administration by different routes and dosage schedules.

Considerable variability in penetration between human samples obtained from adjacent areas of the same leg or between mouse specimens from animals of the same age and genetic background does exist, but these data are typical of the variability in most studies which quantitate percutaneous absorption [11]. This variability, which is greater with the chromatographic procedure, is much greater than the variability of the assay procedures. Both analytical techniques were utilized to determine whether metabolism, either in the skin during penetration or in the well following penetration, could be occurring since the chromatographic method measures only the free FU. The differences in penetration between methods were not statistically significant; however, a few of the individual experiments showed decreases in the cumulative percent penetrated at earlier times using the chromatographic method. This could

§ N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA with 1% TMCS, Pierce Chemical Co., Rockford, Illinois).

TABLE I

Cumulative percent penetration of FU

Time	Human Skin		Mouse Skin	
	LSC[a]	GLC[b]	LSC[a]	GLC[b]
30 min	1.2	1.5	1.1	1.5
1 hr	1.3	2.2	1.8	2.0
2 hr	2.0	2.6	2.9	2.6
3 hr	2.7	3.4	5.4	3.8
6 hr	4.0	3.0	8.5	11.7
16 hr	8.3	5.7	16.7	19.7
24 hr	12.9	6.1	22.6	30.6

0.02 ml of a 4% solution was applied to the epidermis. All human samples were from adjacent areas of the same leg. All values represent a mean of four samples.

[a] Liquid scintillation counting

[b] Gas liquid chromatography

TABLE II

Mean 20-hr penetration data

Samples	Human Skin		Mouse Skin	
	LSC (%)	GLC (%)	LSC (%)	GLC (%)
1	4.3	6.7	23.1	43.0
2	11.3	11.2	11.2	16.4
3	13.1	12.0	33.1	17.3
4	28.1	6.4	26.5	13.5
5	5.3	7.9	32.1	39.7
6	17.2	19.3	—	—
7	6.3	6.7	—	—
8	7.2	13.5	—	—
9	—	40.1	—	—
Mean ± S.D.	11.6 ± 7.5	13.4 ± 10.1	25.2 ± 7.9	25.9 ± 12.6

0.01 ml of a 4% solution was applied to the epidermis. Samples 1–4 were from one leg; samples 5–9 were from another leg.

TABLE III

Mean 20-hr samples expressed as flux per unit time

Sample	Method	Flux* moles/cm²/hr
Human skin	Liquid scintillation	1.09×10^{-8}
Human skin	Gas chromatographic	1.26×10^{-8}
Mouse skin	Liquid scintillation	2.37×10^{-8}
Mouse skin	Gas chromatographic	2.44×10^{-8}

Mean 20-hr data from Table II.

* Skin area exposed to drug was 1.63 cm².

very likely be due to "metabolism" by soluble enzymes in the penetration wells.

The major metabolism of FU takes place intracellularly and involves anabolic changes in the molecule to form 5-fluorouridine (FUR) and 5-fluoro-2'-deoxyuridine-5' monophosphate (FdUMP), which is believed to be the active form of this metabolite [6, 7]. Since the antitumor activity has been related to FdUMP, this may be the critical compound to quantitate in studies of topical FU therapy. In principle, this compound would be too polar to penetrate normal skin effectively, but it could be formed intracellularly after penetration of FU. Since the ^{14}C label would be retained by these metabolites, penetration studies using analytical methods specific for FU, FUR, and FdUMP in normal and diseased skin could be useful in the assessment of the clinical activity of topically applied FU.

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